No new matter is added.

A marked up copy of the amended claims per 37 C.F.R. §1.121 is attached to this response.

FINALITY OF OFFICE ACTION

A Petition removing finality of the previous Office Action has been granted.

THE REJECTION OF CLAIMS 1-8, 10-20 and 25-45 UNDER 35 U.S.C. § 103(a)

Response to Examiner's Comment that Applicant attacked the cited references individually in addressing rejections under 35 U.S.C. §103(a)

Prior to addressing the rejections under 35 U.S.C. §103(a), we note that in the instant Office Action, the Examiner alleges that in the Amendment and Response filed September 10, 2002, Applicant attacked the cited references individually, and that one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references.

It is respectfully submitted that as discussed below and in the previous Amendment, Applicant did not attack the references individually. Rather, Applicant discussed the teachings of each cited reference individually, then demonstrated that the combination of the cited references did not result in the instantly claimed subject matter. In the previous Amendment and in the instant Amendment, Applicant has organized arguments responsive to the rejection under 35 U.S.C. §103(a) as follows:

1) The instant claims.
2) Teachings of each cited reference. Within the discussion of the teachings of each cited reference, this section also sequentially addresses each of the passages of the individual references alleged by the Examiner to teach or suggest the instantly claimed subject matter.
3) Arguments that the combined teachings of the cited references do not lead to the instantly claimed subject matter.

A. REJECTION OF CLAIMS 1-8, 10-20 AND 25-27 UNDER 35 U.S.C. § 103(a) OVER BRENNAN IN VIEW OF CANARD *ET AL.* AND SCHULZ

Claims 1-8, 10-20, and 25-27 are rejected under 35 U.S.C. §103(a) over Brennan (U.S. Patent 5,174,962) in view of Canard *et al.* (U.S. Patent No. 5,798, 210) and further in view of Schulz (U.S. Patent No. 6,232,076 B1) because Brennan allegedly teaches a mass spectrometric method for identifying nucleotides at one or more base positions of target nucleic acid molecules by synthesizing extension products using primers and either mass-matched or pair-matched nucleotides and chain terminating nucleotides, calculating a mass shift from a period for the mass of each extension product, and identifying the nucleotides at each base position that correspond to each mass shift; Canard *et al.* allegedly teaches methods using a plurality of duplex hairpin primers that are ligated to single-stranded templates so that there is periodicity of distribution of the extension products; and Schulz allegedly teaches methods in which the mass-matched deoxynucleotide is deoxyinosine and the chain-terminating nucleotide base pairs are mass-matched and have distinct molecular weights.

It is concluded that it would have been *prima facie* obvious to one of skill in the art at the time the instant application was filed to combine the method of Brennan with the duplex hairpin primers of Canard *et al.* and the mass-matched deoxynucleotide deoxyinosine allegedly taught by Schulz, to arrive at the instantly claimed subject matter. This rejection is respectfully traversed.

Relevant law

In order to set forth a *prima facie* case of obviousness under 35 U.S.C. § 103: (1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (*ACS Hospital Systems, Inc. v. Montefiore Hospital*, 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed. Cir. 1984)) and (2) the combination of the cited references must actually teach or suggest the claimed subject matter. Further, that which is within the capabilities of one skilled in the art is not synonymous with that

which is obvious. *Ex parte Gerlach*, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art" *In re Keller*, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed subject matter, absent some teaching or suggestion supporting the combination (*ACS Hosp. Systems, Inc. v Montefiore Hosp.* 732 F.2d 1572, 1577. 221 USPQ 929, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" *W.L. Gore & Associates, Inc. v Garlock Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

The prior art must provide a motivation whereby one of ordinary skill in the art would have been led to do that which the applicant has done. *Stratoflex Inc. v Aeroquip Corp.*, 713 F.2d 1530, 1535, 218 USPQ 871, 876 (Fed. Cir. 1983). In addition, the mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 USPQ 1783 (Fed. Cir. 1992).

Also, it is impermissible to ignore the advantages, properties, utilities and unexpected results that flow from the claimed invention; they are part of the invention as a whole. *In re Sernaker*, 702 F.2d 989, 217 USPQ 1 (Fed. Cir. 1983). Unexpected properties must always be considered when determining obviousness. A compound's structure and properties are inseparable so that unexpected properties are part of the subject matter as a whole. *In re Papesh*, 315 F.2d 381, 137 USPQ 43 (CCPA 1963).

Analysis

The claims

Claims 1-8

Claim 1 is directed to a method for identifying a nucleotide at one or more base positions in a target nucleic acid molecule, including synthesizing extension products of the target nucleic acid in the presence of chain terminating nucleotides and mass-matched nucleotides; determining the mass of each extension product; and calculating a mass shift from a period for the mass of each extension product, whereby nucleotide(s) at one or more base positions is determined by identifying the nucleotide that corresponds to each mass shift. Claim 5 is directed to a method for identifying a nucleotide at one or more base positions in a plurality of target nucleic acid molecule using steps similar to those set forth in claim 1. Claims dependent on claims 1 and 5 further define mass-matched nucleotides and methods of determining of nucleotide sequences of target nucleic acids.

Definition of mass-matched nucleotides:

As defined in the specification, "mass-matched nucleotides" refers to a set of nucleotide analogs in which each analog is of **identical** mass to each of the other analogs (*see, e.g.*, page 15, line 29 through page 16, line 26 of the specification).

Definition of mass shift:

As disclosed in the specification, a mass shift is a deviation from the corresponding predetermined periodic reference mass. The mass shift is defined as the distance in daltons between the observed peak (*i.e.*, mass) of the extension product and the nearest periodic reference mass peak, which occurs with a periodicity (relative to the reference primer used to generate extension

products) that is equal to the mass of the mass-matched nucleotide or of the pair-matched nucleotide (*see, e.g.*, page 18, lines 25-28 of the specification).

Claims 10-20

Claim 10 is directed to a method for determining a nucleotide sequence of a target nucleic acid molecule, including incorporating pair-matched nucleotides into the target nucleic acid; synthesizing extension products of the target nucleic acid in the presence of a partially duplex hairpin primer, chain terminating nucleotides and pair-matched nucleotides; determining the mass of each extension product; and calculating a mass shift from a period for the mass of each extension product; whereby the nucleotide sequence of the target nucleic acid is determined by assigning a nucleotide corresponding to each mass shift. Claim 13 is directed to a method for determining nucleotide sequences of a plurality of target nucleic acid molecule by using steps similar to those set forth in claim 10. Claims dependent on claims 10 and 13 further define the chain terminating nucleotides and primers.

Claim 17 is directed to a method for detecting one or a plurality of target nucleic acid(s) or one or plurality of nucleotides therein, which includes the steps of copying the target nucleic acid molecule(s) in the presence of a pair-matched set of nucleotides; denaturing the resulting copies of the target(s) to produce single-stranded templates; annealing and ligating one or a plurality of partially duplex hairpin primers to the single-stranded template(s); extending the primer(s) in the presence of chain terminating nucleotides and pair-matched nucleotides to produce extension products, where the extension products follow a periodic mass distribution that is determined by the mass of the pair-matched nucleotide set; and detecting each of the targets or nucleotides therein by virtue of the mass shift of each extension product from its corresponding periodic reference mass. Claims dependent on claim 17 further define the chain terminating nucleotides and primers.

Definition of pair-matched nucleotides:

As defined in the specification, "pair-matched nucleotides" refer to a nucleotide set in which the nucleotide analogs are selected such that the total mass of each base pair is **identical** (*see, e.g.*, page 15, line 29 through page 16, line 26 of the specification).

Claim 27

Claim 27 is directed to a method for detecting a plurality of target nucleic acid molecules in a sample containing nucleic acid molecules, including preparing a composition containing a plurality of pair-matched nucleic acid molecules or mass-matched nucleic acid molecules from a sample comprising the target nucleic acid molecules; analyzing the resulting composition by mass spectrometry; and detecting target nucleic acid molecules.

Claims 25 and 26

Claim 25 is directed to a method for detecting different nucleotide base compositions in a population of nucleic acids having identical length, including synthesizing the nucleic acids in the presence of one or more nucleotide analogs to produce synthesized nucleic acids; and determining a mass of each synthesized nucleic acid; whereby different nucleotide base compositions are detected by determining the mass of each synthesized nucleic acid and the masses of nucleic acids having different base compositions are separated in a predetermined interval by the nucleotide analog(s). Claim 26 is directed to the method of claim 25 in which the population of nucleic acids having identical length and different base compositions differ in base composition by a single base.

Separation of a population of nucleic acids:

As taught in the specification, a population of nucleic acids having the same length but different base compositions can be resolved by synthesizing the nucleic acids in the presence of a nucleotide analog to produce synthesized nucleic acids having incorporated the nucleotide analog, where the nucleotide

analog is selected to optimally separate the masses of the population of nucleic acids according to their individual base compositions (*see, e.g.*, page 10, lines 8-25).

Thus, the instant claims are directed to methods of identifying nucleotides at one or more base positions, sequencing, or detecting target nucleic acids, which involve one or more of the steps of (1) extending primers in the presence of mass-matched nucleotides; (2) extending primers in the presence of pair-matched nucleotides; (3) determining masses of nucleic acid molecules or fragments (*e.g.*, extension products) thereof; (4) adding nucleotide analogs that separate nucleic acids of identical length but different base compositions in a predetermined interval; and (5) determining the identity of a nucleotide at one or more base positions by calculating a mass shift from a periodic reference mass for the mass of each extension product.

Teachings of the cited art and differences from the claimed methods

Summary

The pending claims are directed to methods for analyzing more than one nucleic acid molecule or fragments thereof (*e.g.*, extension products of a nucleic acid template) using appropriately selected nucleotide analogs that separate their masses according to a predetermined interval. The methods of analyses include methods for identifying the nucleotide at one or more base positions in a target molecule; methods for determining the nucleotide sequence of a target nucleic acid; and methods for separating a population of nucleic acids of identical length but different base compositions. In some methods, the nucleotide analogs are mass-matched nucleotides or pair-matched nucleotides, and the masses of the nucleic acid molecules or fragments thereof are analyzed by determining a mass shift of each molecule or fragment from its corresponding nearest periodic reference mass. The periodic distribution of the nucleic acid molecules or fragments thereof is determined by factors such as the mass of the primer that is extended and the mass of the nucleotide analog.

As discussed below, none of the cited references, singly or in any combination, teaches or suggests methods of identifying nucleotides at one or more base positions, sequencing, or detecting target nucleic acids that involve any of the steps of (1) measuring masses of nucleic acids or fragments thereof; (2) using mass-matched nucleotides or pair-matched nucleotides; (3) selecting nucleotide analogs that separate the masses of a mixture of nucleic acids of identical length but different base compositions according to a predetermined interval; or (4) calculating a mass shift from a periodic reference mass for the mass of each extension product.

Brennan

Brennan teaches methods, apparatuses, and reagents for sequencing target nucleic acids. The target nucleic acid to be sequenced is subjected to Sanger sequencing reactions in which each chain-terminated fragment is associated with a base-specific nuclide that is detectable by mass spectrometry. The chain-terminated fragments are separated according to size by any separation method, such as gel electrophoresis; the size-separated fragments are sequentially introduced into a mass spectrometer; and the terminal base of each fragment is identified by mass spectrometric detection of the combustion products (*e.g.*, SO_2, Cl_2 or Br_2) of a nuclide ($^{32}S/^{33}S/^{34}S/^{36}S$ or $^{35}Cl/^{37}Cl/^{79}Br/^{81}Br$) that is uniquely associated with the terminal base. Thus, the masses of the combustion products (and, therefore, the unique base with which each nuclide is associated) are distinguished by the difference in the masses of the nuclide isotopes. The nuclides can be incorporated into the fragments during the A, G, C and T Sanger sequencing reactions by labeling the component primers, the dideoxynucleotides, or the deoxynucleotides with the nuclides, as long as each Sanger sequencing reaction (*i.e.*, A, G, C and T) is uniquely associated with a nuclide label.

Brennan does not teach or suggest methods of identifying nucleotides at one or more positions, sequencing, or detecting target nucleic acid sequences

by measuring the masses of the extension products. Instead, Brennan measures the masses of **the combustion products of a nuclide (i.e., a mass label)** that is incorporated into a nucleic acid sequence. Brennan does not teach or suggest any method involving analysis of masses of nucleic acids or fragments thereof.

Further, there is absolutely no teaching or suggestion in Brennan of mass-matched nucleotides (nucleotides whose masses are identical) or pair-matched nucleotides (masses of base pairs being identical) or of the incorporation of mass-matched nucleotides or pair-matched nucleotides into extension products of target nucleic acids. Brennan merely teaches incorporating nuclide labels into extension products.

Furthermore, Brennan does not teach or suggest the calculation of mass shifts as defined in the instant application. Measuring mass shifts of extension products involves measuring the distance in daltons between the mass peak of the extension product and the nearest periodic reference mass, which occurs with a periodicity (relative to the reference primer used to generate extension products) that is equal to the mass of the mass-matched nucleotide or the pair-matched nucleotide. Since Brennan does teach or suggest mass-matched nucleotides, pair-matched nucleotides, or measurement of masses of extension products, Brennan cannot possibly teach or suggest calculation of mass shifts of extension products.

Brennan merely teaches sequential mass spectrometric identification of the nuclide labels associated with terminal nucleotides in extension products that have already been separated by other means, such as gel electrophoresis. There is no teaching of any modulation of the separation between extension products or of the periodic distribution of extension products that is equal to the masses of the mass-matched nucleotides or pair-matched nucleotides that are incorporated into the extension products.

Additionally, there is no teaching or suggestion in Brennan of methods of detecting mixtures of nucleic acids of equal length but different base

compositions by incorporating nucleotide analogs that separate the masses of the nucleic acids based on predetermined intervals. In fact, Brennan does not teach or suggest separation of masses of nucleic acids at all, much less doing so by incorporating nucleotide analogs.

The Examiner cites passages in Brennan that allegedly teach or suggest the instant methods. Each passage is addressed in turn below.

Abstract

The Examiner alleges that the Abstract of Brennan teaches methods of identifying nucleotides at one or more base positions and determining the nucleotide sequence of a plurality of target nucleic acid molecules by synthesizing extension products in the presence of mass-matched nucleotides, determining masses of extension products, and calculating mass shifts of extension products. The Examiner also alleges that the Abstract teaches methods of determining the nucleotide sequence of a plurality of target nucleic acid molecules by incorporating pair-matched nucleotides into target nucleic acids. The Examiner also alleges that the Abstract inherently teaches a method for detecting different nucleotide base compositions in a population of nucleic acids having identical length and different base compositions by synthesizing the nucleic acids in the presence of nucleotide analogs and determining the mass of each synthesized nucleic acid.

The Abstract of Brennan teaches sequencing methods in which individual nucleotides in the sequences are identified by detecting the **combustion product of a nuclide label** associated with the terminal nucleotide of a nucleic acid sequence.

Contrary to the Examiner's assertions, there is no teaching or suggestion at all of measuring the mass of an extension product or the entire mass of a nucleic acid. The only mass that is measured in Brennan is the mass of the combustion product of a **nuclide label**, not the mass of an extension product.

Since Brennan does not teach or suggest measuring masses of extension

products, it certainly does not teach or suggest calculating the mass shifts of extension products, which involves measuring the distance in daltons between the mass peak of the extension product and the nearest periodic reference mass. There is also no teaching or suggestion in Brennan of using mass-matched nucleotides or pair-matched nucleotides. While Brennan teaches the incorporation of nuclide labels into extension products, Brennan does not teach or suggest the incorporating into extension products of nucleotide analogs whose masses are identical (*i.e.*, mass-matched nucleotides) or the masses of whose base pairs are identical (*i.e.*, pair-matched nucleotides).

There is also no teaching or suggestion in Brennan of separating populations of nucleic acids of identical lengths but different base compositions. While the nucleic acid fragment in Brennan are size separated by, *e.g.*, gel electrophoresis, there is no teaching or suggestion that nucleotide analogs can be used to separate the masses of mixtures of nucleic acids of equal length but different base compositions in a predetermined interval. None of the methods taught or suggested in Brennan involve the steps of synthesizing extension products in the presence of mass-matched or pair-matched nucleotides, determining masses of extension products, calculating mass shifts of extension products, or synthesizing nucleic acids in the presence of nucleotide analogs that are used to separate nucleic acids of identical lengths but different base compositions in a predetermiend interval.

Column 5, line 25, to column 6, line 8

The Examiner alleges that column 5, line 25, to column 6, line 8, of Brennan teaches methods of identifying nucleotides at one or more base positions and of determining the nucleotide sequence of a plurality of target nucleic acid molecules by synthesizing extension products in the presence of mass-matched nucleotides, determining masses of extension products, and calculating mass shifts of extension products. The Examiner also alleges that this passage inherently teaches a method for detecting different nucleotide base

compositions in a population of nucleic acids having identical length and different base compositions by synthesizing the nucleic acids in the presence of nucleotide analogs and determining the mass of each synthesized nucleic acid.

Column 5, line 25, to column 6, line 8, of Brennan teaches sequencing methods in which DNA fragments containing nuclide labels are separated, the nuclide labels are converted *via* combustion to a more convenient species for mass spectrometry determination (*e.g.*, sulfur isotopes are oxidized to sulfur dioxide), and mass spectrometry is used to detect the combustion product of the nuclide label and thereby identify the terminal nucleotide of the DNA fragment.

Contrary to the Examiner's assertions, there is no teaching or suggestion at all in Brennan of measuring the masses of extension products, using mass-matched nucleotides or pair-matched nucleotides, calculating mass shifts, or separating populations of nucleic acids of identical lengths but different base compositions, as noted above. Although this passage teaches that DNA fragments containing nuclide labels are separated, it does not teach or suggest that the fragments are of identical length but different base compositions, and it does not teach or suggest that nucleotide analogs are used to separate the masses of the fragments of identical length but different base compositions in a predetermined interval.

Column 6, line 10, to column 10, line 64

The Examiner alleges that Column 6, line 10, to column 10, line 64 of Brennan teaches methods of identifying nucleotides at one or more base positions and of determining the nucleotide sequence of a plurality of target nucleic acid molecules by calculating mass shifts of extension products whereby nucleotides at one or more base positions in the sequence is determined by identifying the nucleotide that corresponds to each mass shift. The Examiner also alleges that this passage teaches methods of determining the nucleotide

sequence of a plurality of target nucleic acid molecules by incorporating pair-matched nucleotides into target nucleic acids.

Column 6, line 10, to column 10, line 64, of Brennan teaches methods for identifying the individual nucleotides in a DNA sequence by using nuclide markers contained within chain extension fragments. This passage also teaches that chain extension fragments are subjected to combustion so that the combustion products of the nuclide markers can be detected by mass spectrometry. This passage also teaches methods for incorporating nuclide markers into DNA fragments.

Contrary to the Examiner's assertions, there is no teaching or suggestion at all of calculation of mass shifts of extension products or incorporation of pair-matched nucleotides, as noted above. Although this passage teaches that nuclides are incorporated into chain extension fragments, it does not teach or suggest that pair-matched nucleotides are incorporated into the fragments. While this passage teaches that combustion products of nuclide markers (mass labels) are detected by mass spectrometry, it does not teach or suggest that entire masses of nucleic acid molecules or extension products thereof are detected.

Column 11, lines 1-38 and Figures 1 and 2B

The Examiner alleges that Figure 2B of Brennan teaches methods of identifying nucleotides at one or more base positions and determining the nucleotide sequence of a plurality of target nucleic acid molecules by calculating mass shifts of extension products. The Examiner also alleges that column 11, lines 1-38 and Figures 1-2B inherently teach a method for detecting different nucleotide base compositions in a population of nucleic acids having identical length and different base compositions by using nucleotide analogs that separate the masses of nucleic acids having different base compositions in a predetermined interval.

Column 11, lines 1-38, of Brennan describes Figures 1-2B. Figure 1 shows a series of labeled DNA fragments that are size separated by an electrophoresis column. The size separated fragments are then analyzed by mass spectrometry, which detects the combustion products of the nuclide labels that were incorporated into the DNA fragments. Figure 2B shows an "ion current v. time" spectrum of the combustion products of a **nuclide label** associated with a DNA fragment. Because the nuclide label associated with each chain-terminating nucleotide is unique, the sequence of the DNA fragment can be determined.

Contrary to the Examiner's assertions, there is no teaching or suggestion at all of calculation of mass shifts of extension products or separation of nucleic acids of equal length but different base compositions. As noted above, the mass shift is defined as the distance in daltons between the observed peak (*i.e.*, mass) of the extension product and the nearest periodic reference mass. Since Brennan does not teach or suggest any measurement of masses of nucleic acids or extension products thereof (only of a combustion product of a nuclide label associated with a nucleotide in a nucleic acid sequence), there cannot possibly be any teaching or suggestion of a mass shift in the mass measurement of a nucleic acid or fragment thereof.

Further, while the cited passage teaches that DNA fragments containing nuclide labels are separated by an electrophoresis column, it does not teach or suggest that the fragments are of identical length but different base compositions, and it does not teach or suggest that nucleotide analogs are used to separate masses of mixtures of nucleic acids of identical length but different base compositions based on predetermined intervals.

The spectrum in Figure 2B shows the separation of the masses of the peaks of the **combustion products of nuclide labels**. There is no teaching or suggestion of a population of nucleic acids of identical length but different base compositions being separated based on predetermined intervals, analyses of

masses of nucleic acid molecules or extension products thereof, or calculation mass shifts of extension products.

Examples 1-4 and Schemes D and E

The Examiner alleges that the Examples 1-4 and Schemes D and E of Brennan teaches methods of identifying nucleotides at one or more base positions and determining the nucleotide sequence of a plurality of target nucleic acid molecules by synthesizing extension products in the presence of mass-matched nucleotides, determining masses of extension products, and calculating mass shifts of extension products. The Examiner also alleges that this passage inherently teaches a method for detecting different nucleotide base compositions in a population of nucleic acids having identical length and different base compositions by synthesizing the nucleic acids in the presence of nucleotide analogs and determining the mass of each synthesized nucleic acid.

Examples 1-4 and Schemes D and E of Brennan teach synthetic routes for incorporating sulfur and halogen nuclides into the extension products. The combustion products of the sulfur and halogen nuclides are detected by mass spectrometry and are used to identify the sequence of the nucleic acid.

Contrary to the Examiner's assertions, there is no teaching or suggestion at all in the cited passages of methods of identifying nucleotides at one or more base positions or determining the nucleotide sequence of a plurality of target nucleic acid molecules that involves measuring the masses of extension products, using mass-matched nucleotides or pair-matched nucleotides, calculating mass shifts, or separating populations of nucleic acids of identical lengths but different base compositions. While these passages may teach methods of incorporating sulfur and halogen nuclides into the extension products, they do not teach or suggest that mass-matched nucleotides are incorporated into extension products. Further, while these passages may teach that masses of combustion products of nuclides are detected by mass

spectrometry, they do not teach or suggest that masses of nucleic acid molecules or extension products thereof are detected.

Thus, the teachings of Brennan bear no relevance to those of the instantly claimed subject matter. Brennan teaches methods of sequencing nucleic acids in which the combustion products of nuclide labels that are associated with the sequence are detected by mass spectrometry. Brennan does not teach or suggest methods of identifying nucleotides at one or more base positions, sequencing, or detecting target nucleic acids by synthesizing extension products in the presence of mass-matched or pair-matched nucleotides, determining masses of extension products, calculating mass shifts of extension products, or synthesizing nucleic acids in the presence of nucleotide analogs that are used to separate nucleic acids of identical lengths but different base compositions as required by the instantly claimed methods.

Canard *et al.*

Canard *et al.* teaches methods of sequencing a target nucleic acid molecule in which the incorporation of 3'-hydroxy ester derivatives of nucleotides during extension of hairpin primers annealed to a target template results in chain termination. Canard *et al.* further teaches that these 3'-hydroxy esters derivatives are characterized by unique fluorescent labels, one for each nucleotide. Canard *et al.* further teaches that the chain termination reaction is reversible because under certain conditions, *e.g.*, basic conditions, the ester derivative can be hydrolyzed to release the fluorescent label with excitation and emission wavelengths that are unique for a given base. Furthermore, Canard *et al.* teaches that after hydrolysis and release of the fluorescent label, the chain extension can be continued on the same template target nucleic acid molecule to identify the next nucleotide in the sequence. Thus, Canard *et al.* teaches a modification of the Sanger sequencing method in which the same sequencing reaction, rather than a set of four sequencing reactions (one for each

nucleotide), can be used to sequentially identify nucleotides in a target nucleic acid molecule.

The teachings of Canard *et al.* are irrelevant to those of the instantly claimed methods. Canard teaches use of hairpin primers so that basic conditions may be employed for sequential hydrolysis and release of fluorescent labels followed by the extension of the same hairpin primer template to detect the next nucleotide in the sequence. The fact that hairpin primers can be employed in basic conditions has no bearing on the instantly claimed methods, which do not involve hydrolysis under basic or any other conditions of chain terminal 3'-hydroxy ester protecting groups followed by continued chain extension.

Canard *et al.* does not teach or suggest methods of identifying nucleotides at one or more positions, sequencing, or detecting target nucleic acid sequences by extending hairpin primers in the presence of pair-matched nucleotides or mass-matched nucleotides. In fact, Canard *et al.* does not teach or suggest the use of mass-matched nucleotides or pair-matched nucleotides at all. Instead, Canard *et al.* teaches extension of hairpin primers in the presence of fluorescently labelled 3'-hydroxy esters derivatives.

The sequencing method taught by Canard *et al.* does not involve measuring masses of extension products, nor calculation of mass shifts of extension products, which involves measuring the distance in daltons between the mass peak of the extension product and the nearest periodic reference mass. Further, there is no teaching or suggestion in Canard *et al.* of the periodicity of distribution of extension products. As noted above, the periodicity of distribution of the extension products with respect to the reference primer is defined by the masses of either the mass-matched nucleotide or the pair-matched nucleotides that are incorporated in extension products. Since Canard *et al.* does not teach or suggest mass-matched nucleotides or pair-matched nucleotides or the incorporation of said nucleotides into extension products, it

certainly does not teach or suggest periodicity of the distribution of extension products.

There is also no teaching or suggestion anywhere in Canard *et al.* of separation or analysis of a mixture of nucleic acid fragments of identical length, much less doing so by introducing nucleic acid analogs into the fragments where the analogs are selected to separate the fragments of identical length according to differences in base composition in a pre-determined interval.

The Examiner cites passages in Canard *et al.* that allegedly teach or suggest the instant methods. Each passage is addressed in turn below.

Column 21, line 12 to column 22, line 67

The Examiner alleges that column 21, line 12, to column 22, line 67 of Canard *et al.* teaches a method where there is periodicity of the distribution of extension products.

Column 21, line 12, to column 22, line 67 of Canard *et al.* teaches ligation of hairpin primers to templates and the use of these primers with chain terminating nucleotides containing fluorescently labeled protecting groups. The protecting group on the chain terminating nucleotide is released, followed by sequential extension of the hairpin primer to determine the next nucleotide in the sequence.

Contrary to the Examiner's assertions, there is no teaching or suggestion of periodicity of the distribution of extension products in the methods of Canard *et al.* This passage merely teaches ligation of hairpin primers and extension of these primer under basic conditions; there is absolutely no teaching or suggestion of a periodicity of distribution of the masses of extension products because there is no measurement of such masses at all. As noted above, the periodicity of distribution of the extension products with respect to the reference primer is defined by the masses of either the mass-matched nucleotide or the pair-matched nucleotides that are incorporated in extension products. There is no reference to such periodicity in Canard *et al.*

This passage also does not teach or suggest the use of mass-matched nucleotides or pair-matched nucleotides which, as noted above, are used to determine the periodicity of distribution of the extension products. None of the methods taught or suggested in Canard *et al.* involve the steps of extending primers in the presence of mass-matched or pair-matched nucleotides, determining the periodicity of the distribution of extension products, or synthesizing nucleic acids in the presence of nucleotide analogs that are used to separate nucleic acids of identical lengths but different base compositions.

Column 4, line 66 to column 5, line 4

The Examiner alleges that column 4, line 66 to column 5, line 4, of Canard *et al.* provides motivation for combining the hairpins of Canard *et al.* with Brennan to achieve the expressed advantages of using the hairpin primers under basic conditions, namely the repetition of the sequencing reaction without the addition of a primer at each step.

Column 4, line 66, to column 5, line 4 of Canard *et al.* teaches that the use of hairpin primers makes it possible to "use basic conditions for deprotection of the 3' hydroxyl compatible with a repetition of the procedure without addition of a primer at each step of the indirect determination of a nucleotide inserted." Canard *et al.* teaches that by using basic conditions in sequencing reactions, fluorescently labelled protecting groups on the 3' position of chain terminal nucleotides used in the sequencing reaction are selectively removed and identified to detect the terminal nucleotide. The same sequencing template is then subjected to another round of extension reactions without having to add primers.

Contrary to the Examiner's assertions, and as discussed above, the adaptability of the hairpin primer to the sequencing method of Canard *et al.* has no bearing on the instantly claimed methods, which do not involve hydrolysis under basic or any other conditions of chain terminal 3'-hydroxy ester protecting groups followed by continued chain extension of the same sequencing template.

Rather, the instant methods are directed towards the methods of identifying nucleotides at one or more base positions, sequencing, and detecting target nucleic acids that involve extension of primers in the presence of pair-matched nucleotides, mass-matched nucleotides or other suitable nucleotide analog to effect mass separation in a predetermined interval. Since Canard *et al.* does not teach or suggest that their sequencing methods can be used with mass-matched nucleotides or pair-matched nucleotides, Canard *et al.* is not relevant to the instant claims.

Schulz

Schulz teaches methods of reducing the degradation of polymerase chain extension products containing nonradioactive detection moieties by adding a base, buffer, or reducing agent to the extension reaction. Schulz also teaches methods that generate extension products containing nonradioactive detection moieties, such as fluorescently labeled nucleotides. Schulz also teaches that nucleotides that can be used in the polymerase chain extension reactions include deoxyinosine.

Schulz does not teach or suggest that deoxyinosine may be used as a mass-matched nucleotide.

The Examiner cites a passage in Schulz that allegedly teaches or suggests the instant methods. This passage is addressed below.

Column 8, lines 16-36

The Examiner alleges that column 8, lines 16-36, of Schulz teaches a method wherein the chain-terminating nucleotide base pairs are mass-matched and have distinct molecular weights. The Examiner also alleges that this passage teaches a method wherein the mass-matched deoxynucleotide is deoxyinosine.

Column 8, lines 16-36, of Schulz teaches that a polymerase extension product can include the common nucleotides that occur naturally in DNA and

RNA or can include nucleotide analogs such as deoxyinosine monophosphate and 7-deaza-deoxyguanosine monophosphate.

Contrary to the Examiner's assertion, there is no teaching or suggestion of mass-matched nucleotides in Schulz. As noted above, "mass-matched nucleotides" refers to a set of nucleotide analogs in which each analog is of identical mass to each of the other analogs. Although the cited passage teaches deoxyinosine as an alternative nucleotide that can be incorporated during a polymerase chain reaction, this passage does not teach or suggest any methods for identifying nucleotides at one or more base positions in a target nucleic acid or of sequencing a target nucleic acid by incorporating mass-matched or pair-matched nucleotides into extension products of the target so that the masses of the extension products follow a periodic distribution. Nowhere does Schulz teach mass-matched nucleotides or deoxyinosine as a mass-matched nucleotide.

As noted above, Schulz teaches methods of reducing the degradation of polymerase chain extension products containing nonradioactive detection moieties by adding a base, buffer, or reducing agent to the extension reaction. Schulz does not teach or suggest synthesizing extension products in the presence of mass-matched nucleotides or pair-matched nucleotides. Schulz also does not teach or suggest any methods involving determining the masses of extension products or of entire nucleic acids at all. Since Schulz does not teach or suggest determining the masses of extension products, there certainly cannot be any teaching or suggestion of calculating mass shifts of extension products (which involves measuring the distance in daltons between the mass of the extension product and the nearest periodic reference mass), nor of a periodicity of distribution of masses of extension products, where such periodicity is based on the mass of mass-matched nucleotides or pair-matched nucleotides.

Furthermore, there is no teaching or suggestion anywhere in Schulz of methods involving the separation or analysis of a mixture of nucleic acid fragments of identical length, much less doing so by introducing nucleic acid analogs into the fragments where the analogs are selected to separate the fragments of identical length according to differences in base composition in a pre-determined interval.

The combination of teachings of the cited references does not result in the instantly claimed methods.

Claims 1-8

As noted above, claims 1-8 are directed to a method of identifying a nucleotide at one or more base positions in a target nucleic acid molecule by synthesizing extension products in the presence of mass-matched nucleotides; determining the mass of extension products; and calculating mass shifts of extension products from a period. Thus, claims 1-8 involve the step of synthesizing extension products in the presence of mass-matched nucleotides. As discussed above, "mass-matched nucleotides" refers to a set of nucleotide analogs in which each analog is of identical mass to each of the other analogs (*see, e.g.*, page 15, line 29 through page 16, line 26 of instant specification).

The combination of the cited references does not result in the subject matter of the rejected claims.

Brennan teaches methods of sequencing nucleic acids by detecting the masses of combustion products of nuclide labels that were incorporated into extension products. While Brennan teaches the incorporation of nuclide labels into extension products, it does not teach or suggest the incorporation of mass-matched nucleotides into extension products. Brennan does not teach or suggest any modification of its methods to include mass-matched nucleotides. In fact, Brennan does not teach or suggest mass-matched nucleotides at all. Further, there is no teaching or suggestion in Brennan of other elements of the rejected claims such as measuring masses of nucleic acids or fragments thereof, nor of calculating a mass shift from a periodic reference mass for the mass of

each extension product. While Brennan teaches the detection of combustion products of nuclide markers (mass labels) by mass spectrometry, Brennan does not teach or suggest any methods involving the measurement of entire masses of extension products, much less calculating a mass shift from a periodic reference mass for the mass of each extension product.

Canard *et al.*, which uses hairpin primers and protecting groups on chain terminating nucleotides that allow for the release of the protecting group followed by continued extension of the primer, does not cure these deficiencies. There is no teaching or suggestion that the hairpin primers in Canard *et al.* are extended in the presence of mass-matched nucleotides. Canard *et al.* does not teach or suggest any methods involving the synthesis of primer extension products in the presence of mass-matched nucleotides. Canard *et al.* does not teach or suggest any modification of its methods to include mass-matched nucleotides. In fact, Canard *et al.* does not teach or suggest mass-matched nucleotides at all. While Canard *et al.* teaches the step of extending hairpin primers in the presence of fluorescently labelled chain terminal nucleotides under basic conditions, Canard *et al.* does not teach or suggest any method involving the step of extending hairpin primers in the presence of mass-matched nucleotides. Canard *et al.* also does not teach or suggest measuring masses of nucleic acids or fragments thereof, nor of calculating a mass shift from a periodic reference mass for the mass of each extension product. Therefore, the teachings of Canard *et al.* do not cure the deficiencies in Brennan.

Further, Schulz, which teaches primer extension reactions that generate extension products containing nonradioactive detection moieties, also does not cure the deficiencies of Brennan, Canard *et al.*, or their combination. Schulz does not teach or suggest any methods involving the synthesis of primer extension products in the presence of mass-matched nucleotides. Although Schulz teaches that deoxyinosine can be used in polymerase chain reactions, Schulz does not teach or suggest that deoxyinosine may be used as a mass-

matched nucleotide. While Schulz teaches deoxyinosine as an alternative nucleotide that can be incorporated during a polymerase chain reaction, Schulz does not teach the incorporation of nucleotide analogs that are all of identical mass (mass-matched) into the polymerase chain reaction, much less doing so to create a periodic distribution of masses of extension products synthesized by the reaction.

Since neither Brennan, Canard *et al.* nor Schulz teaches or suggests any methods involving the steps of synthesizing extension products in the presence of mass-matched nucleotides, the combination of these references does not result in the claimed methods. Further, none of the references, singly or in combination, teaches the steps of measuring masses of extension products, calculating mass shifts, or determining the periodicity of distribution of extension products as noted above.

Furthermore, none of the references, singly or in combination, teaches or suggests any of elements of the dependent claims, such as the use of particular mass-matched nucleotides or mass-matched nucleotides of identical mass, that are used in conjunction with the methods set forth in the independent claims from which they depend.

Claims 10-20

As noted above, claims 10-20 are directed to a method for determining sequences of nucleotides in target nucleic acids and a method for detecting target nucleic acids involving the step of synthesizing extension products in the presence of pair-matched nucleotides. As noted above, "pair-matched nucleotides" refer to a nucleotide set in which the nucleotide analogs are selected such that the total mass of each base pair is identical (*see, e.g.*, page 15, line 29 through page 16, line 26 of instant specification).

The combination of the cited references does not result in the rejected claims.

Brennan teaches methods of sequencing nucleic acids by detecting the masses of combustion products of nuclide labels that were incorporated into extension products. While Brennan teaches methods of sequencing nucleic acids that involve the incorporation of nuclide labels into extension products, Brennan does not teach or suggest the incorporation of pair-matched nucleotides into extension products. Brennan does not teach or suggest any modification of its methods to include nucleotide analogs in which the total mass of each base pair is identical (*i.e.*, pair-matched nucleotides). In fact, Brennan does not teach or suggest pair-matched nucleotides at all. Thus, while Brennan teaches sequencing methods involving the step of incorporating nuclides into extension products, it does not teach or suggest any methods involving the step of incorporating pair-matched nucleotides into extension products. Further, as discussed above, there is no teaching or suggestion in Brennan of other elements of the rejected claims such as measuring masses of nucleic acids or fragments thereof, nor of calculating a mass shift from a periodic reference mass for the mass of each extension product.

Canard *et al.*, which uses hairpin primers and protecting groups on chain terminating nucleotides that allow for the release of the protecting group followed by continued extension of the primer, does not cure these deficiencies. There is no teaching or suggestion that the primers in Canard *et al.* are extended in the presence of pair-matched nucleotides. Canard *et al.* also does not teach or suggest measuring masses of nucleic acids or fragments thereof, nor of calculating a mass shift from a periodic reference mass for the mass of each extension product. Therefore, the teachings of Canard *et al.* do not cure the deficiencies in Brennan.

Schulz, which teaches primer extension reactions that generate extension products containing nonradioactive detection moieties, also does not cure these deficiencies. Schulz does not teach or suggest any methods involving the synthesis of primer extension products in the presence of pair-matched

nucleotides. While Schulz teaches nucleotides that can be incorporated during a polymerase chain reaction, Schulz does not teach or suggest any method involving the incorporation of nucleotide analogs whose base pairs are all of identical mass (pair-matched) into the polymerase chain reaction so that the masses of the extension products follow a periodic distribution.

Since neither Brennan, Canard *et al.* nor Schulz teaches or suggests any methods involving the step of synthesizing of extension products in the presence of pair-matched nucleotides, the combination of these references does not result in the claimed methods. Additionally, none of the references, singly or in combination, teaches the step of measuring masses of extension products, calculating mass shifts, or determining the periodic mass distribution of extension products as noted above.

Furthermore, none of the references, singly or in any combination, teaches or suggests any of elements of the dependent claims, such as the use of mass-matched chain terminating nucleotides, chain terminating nucleotide base pairs that have distinct molecular weights, or mass-labeled primers, that are used in conjunction with the methods of the independent claims from which they depend.

Claim 27

As noted above, claim 27 is directed to a method of detecting a plurality of target nucleic acids involving the preparation of compositions of pair-matched nucleic acid molecules or mass-matched nucleic acid molecules, and analyzing the resulting compositions by mass spectrometry.

Further, as noted above, neither Brennan, Canard *et al.* nor Schulz teaches or suggests any mass-matched or pair-matched nucleotides, much less analyzing compositions of nucleic acid molecules containing mass-matched or pair-matched nucleotides by mass spectrometry. Therefore, the combination of these references does not result in the method of Claim 27.

Claims 25 and 26

As noted above, claims 25 and 26 are directed to methods of detecting different nucleotide base compositions in a population of nucleic acids by synthesizing nucleic acids in the presence of nucleotide analogs that separate the masses of the synthesized nucleic acids based on pre-determined intervals. As noted above, the nucleotide analog is selected to optimally separate the masses of the population of nucleic acids according to their individual base compositions (*see, e.g.*, page 10, lines 8-25 of instant specification).

The combination of the cited references does not result in the subject matter of the rejected claims.

Brennan teaches methods of sequencing nucleic acids by detecting the masses of combustion products of nuclide labels that were incorporated into extension products. Although Brennan teaches sequencing methods in which labelled nucleic acid fragments are size separated before analysis of the combustion products of the labels by mass spectrometry, Brennan does not teach or suggest that the fragments are of identical length but different base compositions. There is no teaching in Brennan of methods for detecting a plurality of target nucleic acids in a population of nucleic acid fragments of identical length but different base compositions. Further, Brennan does not teach or suggest introducing nucleic acid analogs into the fragments, where the analogs are selected to separate the masses of fragments of identical length but different base composition in a predetermined interval. Brennan merely teaches the determination of the masses of combustion products of nuclide labels, not entire masses of a population of nucleic acids that are separated based on predetermined intervals.

The teachings of Canard *et al.* do not cure the deficiencies in Brennan. Canard *et al.* teaches methods for sequencing nucleic acids that involve preparing base-labile fluorescently labeled protecting groups. Canard *et al.* does not teach or suggest methods of separating a population of nucleic acids of

identical length but different base composition that involves a step of incorporating nucleotide analogs that separate the nucleic acids in a predetermined interval. In fact, there is no teaching or suggestion at all in Canard *et al.* of detecting mixtures of nucleic acids of identical length but different base compositions.

Schulz also does not cure these deficiencies. As noted above, Schulz teaches methods involving incorporating nucleotides into polymerase chain extension reactions. Schulz does not teach or suggest any method that involves the step of incorporating nucleotide analogs into mixtures of nucleic acids of identical length but different base compositions to that the masses of the nucleic acids can be separated based on predetermined intervals.

Since neither Brennan, Canard *et al.* nor Schulz teaches or suggests any method of detecting different base compositions in a population of nucleic acids by synthesizing nucleic acids in the presence of nucleotide analogs that are used to separate masses fragments of the nucleic acids based on pre-determined intervals, the combination of these references does not result in the methods of Claims 25 and 26.

Allegedly "Inherent" Teachings of Brennan with respect to Claims 25 and 26

Furthermore, the Examiner's comment that "Brennan inherently teaches a method for detecting different nucleotide base compositions in a population of nucleic acids having identical length and different base compositions with respect to the difference of a single base" is misplaced. Any discussion of inherency has no place in a consideration of obviousness.

Inherency is not applicable to the question of obviousness.

The concept of inherency is not applicable to the question of obviousness. In re Sporman, 363 F.2d 444, 150 USPQ 449 (CCPA 1965). To refer to an unexpected property or parameter as inherent begs the question of whether the unexpected property rebuts *prima facie* obviousness. The concept of inherency is not properly applicable to the question of obviousness (see, In

re Sporman, 363 F.2d 444, 150 USPQ 449 (CCPA 1965)). Obviousness and inherency are entirely different questions; that which may be inherent is not necessarily known and, therefore, is an indication of unobviousness (In re Sporman, 363 F.2d 444, 449, 150 USPQ 449, 452 (CCPA 1965; see, also In re Naylor, 360 F.2d 765, 152 USPQ 106 (CCPA 1966); In re Adams, 356 F.2d 998, 148 USPQ 742 (CCPA 1966); and In re Shetty, 566 F.2d 81, 195 USPQ 753 (CCPA 1977)). Reference to an unexpected property as inherent, thus, begs the question of whether an unexpected property rebuts *prima facie* obviousness. The claimed structure has never existed so that a property cannot be inherent in what previously did not exist. The unexpected property is part of the invention as a whole, and, therefore, evidence of unobviousness of the claimed subject matter.

In In re Naylor a process for preparing a polybutadiene polymer having unexpected properties was at issue. The CCPA held that the fact that a rubbery polybutadiene having high 1,2-addition might be inherent in following the combined teachings of the prior art is **immaterial**, if one of ordinary skill in the art would not appreciate or recognize that inherent result. In In re Adams, the CCPA held that since properties of a claimed structure are always inherent, it is "transparently erroneous" to state that subject matter cannot be patented on the basis of an inherent property. Finally, in In re Shetty, the court held that "inherency is quite immaterial if, as record established here, one of ordinary skill in the art would not appreciate or recognize that inherent result".

Prior to the instant application the claimed processes never existed either in conception or actuality. Therefore, no property of such process could be inherent, since inherency must flow from what previously existed. The properties with respect to separating different nucleotide base compositions in a population of nucleic acids having identical length according to a predetermined interval are described in the instant application and evidence unobviousness.

Therefore, the fact that the instantly claimed methods possess or exhibit properties not taught by the cited references evidences the unobviousness thereof and the failure to demonstrate *prima facie* obviousness.

B. REJECTION OF CLAIMS 28-45 UNDER 35 U.S.C. § 103(a) OVER BRENNAN IN VIEW OF CANARD *ET AL.*, SCHULZ, AND SHUBER

Claims 28-45 are rejected under 35 U.S.C. §103(a) over Brennan in view of Canard *et al.*, further in view of Schulz and further in view of Shuber (U.S. Patent No. 5,888,778). It is alleged that it would have been *prima facie* obvious to one of skill in the art at the time the instant application was filed to combine Brennan, Canard *et al.* and Schulz as discussed above further with Shuber, which allegedly teaches a single and multiple primer-based method for detecting a mutation in a target nucleic acid sequence, to arrive at the claimed subject matter. This rejection is respectfully traversed.

Relevant law

The relevant law is described above.

Analysis

The claims

Claim 28 is directed to a process for detecting a mutation in a target sequence in a target nucleic acid molecule involving hybridizing a primer that is complementary to a sequence in the target nucleic acid that is adjacent to the region suspected of containing a mutation and where the target nucleic acid may or may not be immobilized; contacting the hybridized primer with a composition containing mass-matched deoxyribonucleoside triphosphates and a chain terminating nucleotide selected from a dideoxyribonucleoside triphosphate or a 3'-deoxynucleoside triphosphate and optionally one or more deoxyribonucleoside triphosphates, such that the hybridized primer is extended until a chain terminating nucleotide is incorporated; and determining the mass of the extended primer, thereby determining whether a mutation is present in the target nucleic acid sequence. Claim 31 is directed to a process that follows the steps of Claim 28, except that a plurality of target nucleic acid sequences are

analyzed. Claims dependent on claims 28 and 31 further describe the chain-terminating nucleotides and mass of the primers.

Claims 34, 36, 38, and 42, and claims dependent thereon are directed to methods for detecting target nucleic acid sequences by preparing single-stranded or double-stranded extension products using mass-matched nucleotides and selectively cleavable primer(s) to target nucleic acids, selectively cleaving the primers, and detecting the cleaved extension products.

Claims 40 and 41 are directed to methods that follows the steps set forth in Claim 38, except the claims additionally include pair-matched nucleotides and analysis of a plurality of target nucleic acid sequences.

Thus, the claims are directed to methods of detecting mutations in target nucleic acids or methods of detecting target nucleic acids which involve the step of extending primers in the presence of mass-matched nucleotides or pair-matched nucleotides.

Teachings of the cited art and the differences from the claimed methods

Brennan, Canard *et al.*, and Schulz

The teachings of Brennan, Canard *et al.*, and Schulz are discussed above.

As noted above, Brennan, Canard *et al.*, and Schulz do not teach or suggest methods involving extending primers in the presence of mass-matched nucleotides or pair-matched nucleotides. In addition, Brennan, Canard *et al.*, and Schulz do not teach or suggest methods involving the detection of mutations in target nucleic acids.

Shuber

Shuber is directed to high-throughput screening methods for detecting and identifying genetic mutations or the presence of disease-causing microorganisms in a biological sample. In Shuber, single-base extensions are carried out by using segmented primers that bind to a template sequence in a cooperative fashion to eliminate false priming and by using labelled nucleotides. Shuber teaches that one of the segmented primers is immediately upstream of a

genetic alteration and that extension of this primer using labelled nucleotides followed by detection of the labels provides for the detection and identification of genetic alterations. Shuber teaches that the second primer is non-extendible.

Shuber does not teach or suggest methods of detecting mutations in target nucleic acids or methods of detecting target nucleic acids that involve the step of extending a primer or multiple primers hybridized to the target in the presence of mass-matched nucleotides or pair-matched nucleotides. Although Shuber teaches that genetic mutations are detected by detecting labelled nucleotides, Shuber does not teach or suggest that mutations can be detected by detecting masses of cleaved extension products.

The Examiner cites passages in Shuber that allegedly teach the instant methods. The passages are discussed in turn below.

Abstract

The Abstract of Shuber teaches high-throughput screening methods that can be used to screen for the presence of genetic alterations and disease-causing microorganisms in biological samples.

The abstract does not teach or suggest the use of mass-matched nucleotides or pair-matched nucleotides in the methods disclosed in Shuber.

Column 7, lines 38-59

The Examiner alleges that column 7, lines 38-59, of Shuber teaches methods of detecting mutations in nucleic acids by hybridizing a single primer or plurality of primers to a target nucleic acid and extending the hybridized primer in the presence of mass-matched deoxyribonucleoside triphosphates and chain terminating nucleotides. The Examiner also alleges that because this passage teaches that the methods of Shuber can be used to detect and identify a target nucleic acid that is available in a small proportion in a sample, there would be motivation to combine the method of detecting mutations in Shuber with the mass spectrometric method of Brennan.

Column 7, lines 38-59, of Shuber teaches that segmented primers can be used to detect genetic mutations in target nucleic acids by using primers that are complementary to a sequence in the target nucleic acid that is adjacent to the region suspected of containing a mutation sequence. This passage also teaches that the methods of detecting mutations disclosed in Shuber can be used to identify target nucleic acids available in small proportions in a sample that would normally have to be amplified.

The applicability of the methods of Shuber for the detection of target nucleic acids that are present in small proportions in sample has no bearing on the instant methods because Shuber does not teach or suggest any method involving extension of hybridized primers in the presence of mass-matched nucleotides or pair matched nucleotides. In fact, Shuber does not teach or suggest mass-matched nucleotides or pair matched nucleotides at all.

Assuming arguendo that there was motivation to combine the teachings of Shuber and Brennan, their combination nevertheless does not result in the instantly claimed methods, as discussed below.

Claims 1, 10, and 13

The Examiner alleges that claims 1, 10, and 13 of Shuber teach methods of detecting mutations in nucleic acids by hybridizing a single or plurality of primers to a target nucleic acid and extending the hybridized primer in the presence of mass-matched deoxyribonucleoside triphosphates and chain terminating nucleotides.

Claims 1, 10, and 13 of Shuber teach methods for screening genomic nucleic acid samples to identify mutations by hybridizing segmented primers to nucleic acids wherein one primer is non-extendible, extending the hybridized primer in the presence of nucleotides, and determining the sequence of the extended primer.

Contrary to the Examiner's assertion, claims 1, 10, and 13 do not teach or suggest methods of detecting mutations that involve the step of extending

primers in the presence of mass-matched nucleotides or pair-matched nucleotides as claimed in the instant methods. In fact, Shuber does not teach or suggest mass-matched nucleotides or pair-matched nucleotides at all. The claims of Shuber have no bearing on the instant methods which employ mass-matched nucleotides, pair-matched nucleotides, determining masses of extended primers, and detecting cleaved extension products.

The instant application teaches that in a primer extension reaction using mass-matched or pair-matched nucleotides, incorporation of a chain terminator will indicate the presence of a mutation or provide information about the target sequence by a shift in periodicity from a periodic reference mass (*i.e.*, mass shift) whose is value is equal to that of a mass-matched or pair-matched nucleotide. Not only does Shuber not teach or suggest methods involving mass-matched nucleotides or pair-matched nucleotides, there is no teaching or suggestion in Shuber that mutations or other sequence alterations can be detected by the masses of the extension products or fragments thereof or by a deviation in periodicity of the masses of the cleaved extension products. Shuber does not teach or suggest modification of its methods to include mass-matched nucleotides or pair-matched nucleotides.

The combination of teachings of the cited references does not result in the instantly claimed methods

As noted above, claims 28, 31, and claims dependent thereon are directed to methods of detecting mutations in target nucleic acid sequences that involve the step of extending primers in the presence of mass-matched nucleotides. Claims 34, 36, 38, 40, 42 and claims dependent thereon are directed to methods of detecting target nucleic acids that involve the step of extending primers in the presence of mass-matched nucleotides or pair-matched nucleotides.

The combination of the cited references does not result in the rejected claims.

Brennan teaches methods of sequencing nucleic acids by detecting the masses of combustion products of nuclide labels that were incorporated into extension products. As noted by the Examiner and as stated above, Brennan does not teach or suggest methods of detecting mutations. Additionally, as noted above, Brennan does not teach or suggest methods involving the step of extending primers in the presence of mass-matched nucleotides or mass-matched nucleotides. Therefore, Brennan does not appear to be relevant to claims 28-45.

Canard *et al.*, which uses hairpin primers and protecting groups on chain terminating nucleotides that allow for the release of the protecting group followed by continued extension of the primer, does not cure these deficiencies. As noted by the Examiner and as noted above, Canard *et al.* does not teach or suggest methods of detecting mutations. Additionally, as noted above, Canard *et al.* does not teach or suggest any methods involving extending primers in the presence of mass-matched nucleotides or pair-matched nucleotides. Therefore, Canard *et al.* does not appear to be relevant to claims 28-45.

Schulz, which teaches primer extension reactions that generate extension products containing nonradioactive detection moieties, also does not cure these deficiencies. As noted by the Examiner and as noted above, Schulz does not teach or suggest methods of detecting mutations. Additionally, as noted above, Schulz does not teach or suggest any methods involving extending primers in the presence of mass-matched nucleotides. Therefore, Schulz does not appear to be relevant to claims 28-45.

Shuber, which teaches methods of detecting and identifying genetic mutations by performing single base extensions of segmented primers that are located immediately upstream of a genetic alteration, also does not cure these deficiencies. Although Shuber teaches extension of segmented primers that are hybridized to target nucleic acids, Shuber does not teach or suggest extending the hybridized primers in the presence of mass-matched nucleotides or pair-

matched nucleotides. Shuber also does not teach or suggest modification of its methods to include mass-matched nucleotides or pair-matched nucleotides. In fact, Shuber does not teach or suggest mass-matched nucleotides or pair-matched nucleotides **at all.**

Since neither Brennan, Canard *et al.*, Schulz, nor Shuber teaches or suggest any methods, including methods of detecting mutation in target nucleic acids or methods of detecting target nucleic acids, that involve the steps of extending primers in the presence of mass-matched nucleotides or pair-matched nucleotides, the combination of these references does not result in the claimed methods. Additionally, none of the cited references, singly or in any combination, teaches or suggests any of the other elements of the claims such as mass-matched chain terminating nucleotides, determining masses of nucleic acid molecules or extension products thereof, or detecting masses of cleaved extension products by mass spectrometry.

REBUTTAL TO ARGUMENTS SET FORTH BY THE EXAMINER IN RESPONSE TO APPLICANT'S TRAVERSAL IN THE AMENDMENT FILED SEPTEMBER 10, 2002

1) The Examiner alleges that in the Amendment and Response filed September 10, 2002, Applicant attacked the cited references individually, and that one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references.

It is respectfully submitted that Applicant did not attack references individually in rebuttal of the rejection set forth under 35 U.S.C. § 103(a). Rather, Applicant systematically (i) distinguished the teachings of each of the cited references from the instantly claimed subject matter; and (ii) showed that the deficiencies of each of the cited references against the claimed subject matter was not cured by any of the other cited references. Applicant then pointed out that none of the references, singly or in any combination, taught or suggested the claimed subject matter.

The Examiner is referred to page 22 of the Amendment and Response filed September 10, 2002, in which the section entitled:

"The combination of teachings of the cited references does not result in the instantly claimed methods"

discusses how the combination of the cited references does not result in the claimed subject matter because, among other reasons of record in the aforementioned Amendment, since all the cited references lack a teaching or suggestion of the instantly claimed elements of measuring the masses of extension products, incorporating mass-matched or pair-matched nucleotides into extension products, generating a periodic distribution of the masses of the extension products, determining a mass shift that identifies nucleotides at one or more positions in a target sequence, or separating a population of nucleic acid molecules of identical length according to their base composition by incorporating nucleotide analogs into the population such that the separation according to base composition occurs in a pre-determined interval, none of the cited references can cure deficiencies in the others. Thus, the cited references, singly or in any combination thereof, fail to teach or suggest the missing elements of the claims.

2) The Examiner asserts that Applicant's argument that there is no motivation to combine the references is not persuasive, particularly in view of Canard *et al.*, which allegedly states "The use of hairpin primer makes it possible to use basic conditions for deprotection of a primer at each step of indirect determination of a nucleotide inserted" and that the "rehybridization of the primer occurs intramolecularly and immediately (Column 4, line 66 to column 5, line 4)." The Examiner alleges that this logic is applicable to all other motivations to combine the references.

Canard *et al.* discusses the use of a hairpin primer that provides for deprotection of the chain terminating nucleotide of the primer under basic conditions during primer extension reactions. The sequencing method taught by Canard *et al.* employs hairpin primers for the extension reactions because the use of hairpin primers makes it possible to use "basic conditions for hydrolysis of the 3'-hydroxyl esters that is compatible with a repetition of the [chain

extension] procedure without addition of a primer at each step of the indirect determination of a nucleotide inserted." (column 4, line 67 to column 5, line 3) **The context in which hairpin primers are taught in Canard *et al.* bears no relevance to the instantly claimed subject matter because the primers in Canard *et al.* are not extended in the presence of mass-matched nucleotides or pair-matched nucleotides and are not used to separate nucleotides of identical length but different base compositions based on pre-determined levels.** The primers in Canard *et al.* are not extended in the presence of mass-matched nucleotides to detect genetic mutations.

Similarly, Brennan, Shuber, and Schulz bear no relevance to the instant claims because they do not teach or suggest methods involving the use of mass-matched nucleotides, pair-matched nucleotides, or nucleotide analogs that separate nucleic acids of identical length but different base compositions according to a predetermined interval.

The instant claims are directed to methods of identifying nucleotides at one or more base positions, sequencing, or detecting target nucleic acids that involve the use of mass-matched nucleotides, pair-matched nucleotides, or nucleic acid analogs that separate nucleic acids of identical length but different base compositions.

As discussed above, Brennan measures combustion products of nucleotides, not the masses of extension products. Brennan does not teach or suggest methods involving the use of mass-matched nucleotides, pair-matched nucleotides, or nucleic acid analogs that separate nucleic acids of identical length different base compositions. Therefore, Brennan is unrelated to the instant teachings. Canard *et al.* teaches sequencing methods involving the use of hairpin primers so that it allows for the sequential release of the protecting group followed by continued extension of the primer under basic conditions. Canard *et al.* does not teach or suggest methods that involve the use of mass-matched nucleotides, pair-matched nucleotides, or nucleotide analogs that

separate nucleic acids of identical length different base compositions. Therefore, Canard *et al.* is of no relevance to the instant teachings.

Schulz teaches primer extension reactions that generate extension products containing nonradioactive detection moieties. Schulz uses deoxyinosine as an alternative nucleotide for polymerase chain reactions but does not teach or suggest deoxyinosine as a mass-matched nucleotide. Schulz does not teach or suggest methods involving the use of mass-matched nucleotides or pair-matched nucleotides in the polymerase chain reactions. Schulz does not teach or suggest methods involving the use of nucleic acid analogs that separate nucleic acids of identical length different base compositions. Therefore, Schulz also bears no relevance to the instant teachings.

Shuber teaches methods that involve the use of segmented primers to detect genetic mutations but does not teach or suggest methods that involve the extension of primers in the presence of mass-matched nucleotides or pair-matched nucleotides.

None of the cited references are related in any way to the instant teachings since the references do not teach or suggest methods of identifying nucleotides at one or more base positions in target nucleic acids, methods of sequencing target nucleic acids, methods of detecting target nucleic acids, or methods of detecting mutations in target nucleic acids that involve the synthesis of extension products or primers in the presence of mass-matched nucleotides or pair-matched nucleotides or involve the use of nucleotide analogs that separate nucleic acids of identical length different base compositions. Because all of the cited references are missing not only any method of analysis of mixtures of nucleic acids or extension products thereof as instantly claimed, but also critical elements of the instant claims as discussed above, their combination cannot possibly lead to the claimed subject matter.

Because the combined references do not teach or suggest the instantly claimed methods, the combined references do not constitute a 35 U.S.C. § 103(a) bar to the instant claims.

* * *

In view of the above, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Cantor *et al.*

Serial No.: 09/880,988

Confirmation No.: 5954

Filed: : June 13, 2001

For: *USE OF NUCLEOTIDE ANALOGS IN THE ANALYSIS OF OLIGONUCLEOTIDE MIXTURES AND IN HIGHLY MULTIPLEXED NUCLEIC ACID SEQUENCING*

Art Unit: 1634

Examiner: Chakrabarti, Arun K.

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Jonathan Ong

MARKED UP CLAIMS (37 C.F.R. § 1.121)

Please amend claims 5 and 28 as follows:

5. (Amended) A method for identifying nucleotides at one or more base positions in a plurality of target nucleic [acids]acid molecules, comprising:

synthesizing extension products of the target nucleic acid in the presence of chain terminating nucleotides and mass-matched nucleotides;

determining the mass of each extension product; and

calculating a mass shift from a period for the mass of each extension product,

whereby the nucleotides in the target nucleic acid molecules are identified by determining the nucleotide that corresponds to each mass shift.

28. (Twice Amended) A process for detecting a mutation in a target nucleic acid sequence in a target nucleic acid molecule, in a sample, comprising:

a) hybridizing a primer to nucleic acid molecules in the sample, thereby producing a hybridized primer and a molecule from the sample, wherein:

the nucleic acid molecules from the sample are optionally immobilized and

the primer is complementary to a sequence in the target nucleic acid sequence that is adjacent to the region suspected of containing a mutation sequence;

b) contacting the hybridized primer with a composition comprising mass-matched deoxyribonucleoside triphosphates and a chain terminating nucleotide selected from a dideoxyribonucleoside triphosphate or a 3'-deoxynucleoside triphosphate and optionally one or more deoxyribonucleoside triphosphates, such that the hybridized primer is extended until a chain terminating nucleotide is incorporated, thereby producing an extended primer; and

c) determining the mass of the extended primer, thereby determining whether a mutation is present in the target nucleic acid sequence.